

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
20 November 2008 (20.11.2008)

PCT

(10) International Publication Number  
**WO 2008/140455 A1**

(51) International Patent Classification:  
*C12N 5/06* (2006.01) *C12N 5/16* (2006.01)

(21) International Application Number:  
PCT/US2007/011559

(22) International Filing Date: 15 May 2007 (15.05.2007)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant (for all designated States except US): **TANOX, INC.** [US/US]; 10301 Stella Link Road, Houston, TX 77025-5497 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **FUNG, Sek, Chung** [US/US]; 3511 Deal, Houston, TX 75025 (US). **MOYLE, Matthew** [US/US]; 3650 Meadow Lake Lane, Houston, TX 77027 (US).

(74) Agent: **BENN, Jacqueline**; Jones Day, 222 East 41st Street, New York, NY 10017 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

(54) Title: TREATMENT OF RADIATION AND CHEMO-THERAPY INDUCED FIBROSIS USING NOVEL ANTI-IL 13 MONOCLONAL ANTIBODIES

(57) Abstract: The present application relates to the treatment of fibrosis using novel anti-IL13 monoclonal antibodies. The antibodies useful in the present invention comprise anti-IL13 antibodies that bind specifically and with high affinity to IL13. In particular, such treatment may be in subjects undergoing radiotherapy and/or chemotherapy. Examples of anti-IL13 antibodies are 228B/C-1, 228A-4, 227-26, and 227-43.

WO 2008/140455 A1

## **TREATMENT OF RADIATION AND CHEMO-THERAPY INDUCED FIBROSIS USING NOVEL ANTI-IL13 MONOCLONAL ANTIBODIES**

### **BACKGROUND**

**[Para 1]** IL13 is a pleiotropic Th2 cytokine produced predominantly by CD4+ T-helper type 2 cells, as well as NKT cells, basophils, and mast cells (Hershey, GKK, J Allergy Clin Immunol. (2003) 111: 677-90). In addition to its known etiologic roles in asthma, IL13 also manifests functions on nonhematopoietic cells, such as smooth muscle cells, epithelial cells, endothelial cells and fibroblast cells. IL13 enhances proliferation and cholinergic-induced contractions of smooth muscles (Wills-Karp, J. Allergy Clin. Immunol., 107: 9 (2001). In epithelial cells IL13 is a potent inducer of chemokine production (Li et al., J. Immunol., 162: 2477 (1999), alters mucociliary differentiation (Laoukili et al., J. Clin. Invest., 108: 1817 (2001), decreases ciliary beat frequency of ciliated epithelial cells (Laoukili et al., J. Clin. Invest., 108: 1817 (2001), and results in goblet cell metaplasia (Zhu et al., J. Clin. Invest., 103: 779 (1999), Grunig et al., Science, 282: 2261 (1998)).

**[Para 2]** IL13 is also known to play a role in fibrosis by promoting fibroblast proliferation, collagen synthesis, and extracellular-matrix remodeling (Wynn TA et al. Nat Rev Immunol. 2004; 4: 583-94). Although TGF- $\beta$  is also involved in fibrosis, its activity is regulated upstream by IL13. In addition, IL13 can directly induce fibrosis via a TGF $\beta$ -independent pathway. Fibrosis is manifested by a loss of pliability and flexibility of the soft tissues down to the muscle layers. Deep tissue fibrosis includes muscle, investing fascia, and all the connective tissue down to bone. Associated functional deficits vary according to the anatomic site involved. Pain, neuropathy, loss of joint range of motion, and distal lymphoedema are often associated with fibrosis. These symptoms and impairments may in turn result in activity limitation, such as difficulty with daily living, including self care, eating, speech and mobility.

**[Para 3]** Fibrosis is a common sequela of radiotherapy treatment for cancer. Patients with head and neck or breast cancer and those with soft tissue sarcoma of the musculoskeletal system frequently experience subcutaneous fibrosis after radiotherapy. Bentzen et al. (Radiother Oncol. 1989; 15: 267-4) have shown that the latency of fibrosis is between 1-2 years postradiotherapy and the severity of fibrosis progresses over time. Van der Kogel et al., in Stell G (ed): Basic clinical radiology (ed 2). Arnold, 1997(p32), estimates that there is a 50% risk of developing clinically detectable fibrosis with conventional external-beam radiotherapy doses.

**[Para 4]** The toxicity of fibrosis limits the use of high dose radiation and chemo-therapy and also increases the rate of morbidity and mortality of cancer patients after completion of therapy. Fibrosis-related toxicity of chemotherapy is frequently dose dependent. The agents associated with the highest incidence of interstitial pneumonitis/fibrosis (ranging 10-40% of treated patients) include bleomycin, busulfan, carmustine and mitomycin C. Pulmonary fibrosis usually occur between 2 to 12 months after the completion of chemotherapy. About 1-2% of patients developing severe pulmonary fibrosis die.

**[Para 5]** Currently, there are no proven medications to prevent or treat fibrosis in cancer patients undergoing radiotherapy and chemotherapy (Abid SH et al., Curr Opin Oncol. (2001) 13: 242-8; Nieder C et al., Anti-cancer Res. (2003) 23: 4991-8).

**[Para 6]** This invention describes a new approach to treat and prevent fibrosis in particular in patients undergoing radiotherapy and/or chemotherapy by inhibiting IL13 induced fibrosis.

#### SUMMARY OF THE INVENTION

**[Para 7]** The present application relates to the treatment of IL13-induced fibrosis using novel anti-IL13 monoclonal antibodies. The antibodies useful in the present invention comprise anti-IL13 antibodies that bind specifically and with high affinity to IL13. In particular, such treatment may be in subjects undergoing radiotherapy and/or chemotherapy. Examples of anti-IL13 antibodies are 228B/C-1, 228A-4, 227-26, and 227-43. The hybridomas that produce these antibodies were deposited on November 20, 2003, with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, under Accession Numbers PTA-5657, PTA-5656, PTA-5654, and PTA-5655, respectively.

**[Para 8]** The present invention includes a method of preventing or reducing cell proliferation, cell activation or extracellular matrix production induced by IL13 in a mammal comprising administering to the mammal a composition comprising an anti-IL13 antibody in an amount sufficient to reduce IL13 mediated cell proliferation or extracellular matrix production.

**[Para 9]** Another embodiment includes a method of treating fibrosis in a patient undergoing chemotherapy and/or radiotherapy comprising administering an anti-IL13 antibody in an amount sufficient to reduce fibrosis in said patient.

**[Para 10]** Another embodiment includes a method for improving radiotherapy and/or chemo-therapy comprising reducing cell proliferation, cell activation or extracellular matrix production induced by IL13 in a mammal comprising administering to the mammal a composition comprising an anti-IL13 antibody in combination with a pharmaceutically

acceptable delivery vehicle, in an amount sufficient to prevent or reduce IL13 mediated cell proliferation, cell activation or extracellular matrix production and allow higher doses of radiotherapy and/or chemotherapy.

**[Para 11]** Another embodiment includes a method of reducing the morbidity or mortality associated with fibrosis in patients undergoing chemotherapy and/or radiotherapy comprising administering an anti-IL13 antibody in an amount sufficient to reduce fibrosis in said patients.

**[Para 12]** The anti-IL13 antibody embodiments include intact (full length) antibodies as well as antibody fragments. The anti-IL13 antibodies useful in the present invention may be chimeric, humanized or human. Antigen-binding antibody fragments of the antibodies include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, and disulfide-linked Fvs (sdFv). The invention also includes single-domain antibodies comprising either a VL or VH domain.

**[Para 13]** The invention also encompasses the use of a composition comprising any one of the anti-IL13 antibodies of the above embodiments, and a carrier, in the methods of the present invention.

**[Para 14]** Another embodiment includes a method of preventing, alleviating or reducing fibrosis in a subject, comprising administering a therapeutically effective amount of an anti-IL13 antibody to a subject, such as in patients undergoing radiotherapy and/or chemotherapy.

**[Para 15]** Another embodiment includes a method of reducing fibrosis by administering an anti-IL13 antibody prior to, in combination with radiotherapy and chemotherapy, and/or after radiotherapy or chemotherapy. Chemotherapeutic agents include, but are not limited to, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside, Cyclophosphamide, Thiotepa, Busulfan, Cytosine, Taxol, Methotrexate, Cisplatin, Melphalan, Vinblastine, bleomycin and Carboplatin.

**[Para 16]** In a further aspect, the invention provides an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an anti-IL13 antibody of the above embodiments, and further comprising a package insert and/or label indicating that the composition can be used to prevent or reduce fibrosis.

#### BRIEF DESCRIPTION OF THE FIGURES

**[Para 17]** Figure 1 shows the dose-dependent binding of anti-IL13 mAbs 228B/C-1, 228A-4, 227-26, 227-43, and the negative control in ELISA.

#### DETAILED DESCRIPTION

**[Para 18]** This invention is not limited to the particular methodology, protocols, cell lines, vectors, or reagents described herein because they may vary. Further, the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope useful in the present invention. As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise, e.g., reference to "a host cell" includes a plurality of such host cells.

**[Para 19]** Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of the invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice useful in the present invention, the methods, devices, and materials are described herein.

**[Para 20]** All patents and publications mentioned herein are incorporated herein by reference to the extent allowed by law for the purpose of describing and disclosing the proteins, enzymes, vectors, host cells, and methodologies reported therein that might be used with the present invention. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

#### ANTIBODY GENERATION

**[Para 21]** The antibodies useful in the present invention may be generated by any suitable method known in the art. The antibodies may comprise monoclonal antibodies. In preparing monoclonal antibodies, recombinant IL13 was used to immunize mice to generate the hybridomas that produce the monoclonal antibodies useful in the present invention. Recombinant IL13 is commercially available from a number of sources (see, e.g. R & D Systems, Minneapolis, MN, PeproTech, Inc., NJ, and Sanofi Bio-Industries, Inc., Tervose, PA.). Alternatively, a gene or a cDNA encoding IL13 may be cloned into a plasmid or other expression vector and expressed in any of a number of expression systems according to methods well known to those of skill in the art. Methods of cloning and expressing IL13 and the nucleic acid sequence for IL13 are well known (see, for example, U.S. Patent No. 5,652,123).

**[Para 22]** The immunogen IL13 polypeptide may, when beneficial, be expressed as a fusion protein that has the IL13 polypeptide attached to a fusion segment. The fusion segment often aids in protein purification, e.g., by permitting the fusion protein to be isolated and purified by affinity chromatography. Fusion proteins can be produced by culturing a recombinant cell transformed with a fusion nucleic acid sequence that encodes

a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of the protein. Fusion segments may include, but are not limited to, immunoglobulin Fc regions, glutathione-S-transferase,  $\beta$ -galactosidase, a poly-histidine segment capable of binding to a divalent metal ion, and maltose binding protein. A fusion protein comprising a mutant form of human IL13 may also be used to generate monoclonal antibodies. In particular, a single mutation resulting in an inactive form of the protein was reported by Thompson et al., J. Biol. Chem. 274: 2994 (1999).

**[Para 23]** In order to generate neutralizing antibodies with high affinity, a fusion protein comprising the IL13 protein fused to an immunoglobulin Fc, such as IgG1, was expressed in a mammalian cell line such that the recombinant protein was naturally glycosylated. The Fc portion of the fusion protein may provide a conformational structure that expose a key epitopes. The glycosylation may also increase the immunogenicity of the epitope, allowing the generation of antibodies to this particular epitope.

**[Para 24]** In generating anti-IL13 antibodies, an immunogen as described above may be administered to various host animals including, but not limited to, a mouse, a humanized mouse, a knock-out mouse, a mouse with a human immune system, hamster, donkey, sheep, goat, guinea pig, horse, chicken, rabbit, camel or any other appropriate host animal, to induce the production of sera containing polyclonal antibodies specific for the antigen. The administration of the immunogen may entail one or more injections of an immunizing agent and, if desired, an adjuvant. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Additional examples of adjuvants which may be employed include the MPL-TDM adjuvant (monophosphoryl lipid A, synthetic trehalose dicorynomycolate). Immunization protocols are well known in the art in the art and may be performed by any method that elicits an immune response in the animal host chosen. Adjuvants are also well known in the art.

**[Para 25]** Typically, the immunogen (with or without adjuvant) is injected into the mammal by multiple subcutaneous or intraperitoneal injections, or intramuscularly or through IV. Depending upon the nature of the immunogen (i.e., percent hydrophobicity, percent hydrophilicity, stability, net charge, isoelectric point etc.), it may be useful to conjugate the immunogen to a protein known to be immunogenic in the mammal being

immunized. Such conjugation includes either chemical conjugation by derivatizing active chemical functional groups to both the immunogen and the immunogenic protein to be conjugated such that a covalent bond is formed, or through fusion-protein based methodology, or other methods known to the skilled artisan. Examples of such immunogenic proteins include, but are not limited to, keyhole limpet hemocyanin, ovalbumin, serum albumin, bovine thyroglobulin, soybean trypsin inhibitor, and promiscuous T helper peptides. Various adjuvants may be used to increase the immunological response as described above.

**[Para 26]** Monoclonal antibodies may be prepared using hybridoma technology, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975) and U.S. Pat. No. 4,376,110, by Harlow, et al., *Antibodies: A Laboratory Manual*, (Cold spring Harbor Laboratory Press, 2<sup>nd</sup> ed. (1988), by Hammerling, et al., *Monoclonal Antibodies and T-Cell Hybridomas* (Elsevier, N.Y., (1981)), or other methods known to the artisan. Other examples of methods which may be employed for producing monoclonal antibodies include, but are not limited to, the human B-cell hybridoma technique (Kosbor et al., 1983, *Immunology Today* 4:72; Cole et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies And Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated in vitro or in vivo.

**[Para 27]** Generally, in making antibody-producing hybridomas, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986), pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine or human origin. Typically, a rat or mouse myeloma cell line is employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), substances that prevent the growth of HGPRT-deficient cells.

**[Para 28]** Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, Calif. and the American Type Culture Collection, Manassas, Va. Human myeloma and mouse-human heteromyeloma cell lines may also be used for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., New York, (1987) pp. 51-63).

**[Para 29]** The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the IL13. The binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by, e.g., immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoadsorbant assay (ELISA). Such techniques are known in the art and within the skill of the artisan. The binding affinity of the monoclonal antibody to IL13 can, for example, be determined by a Scatchard analysis (Munson et al., *Anal. Biochem.*, 107:220 (1980)).

**[Para 30]** After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *supra*). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640. The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium by conventional immunoglobulin purification procedures such as, e.g., protein A-sepharose, hydroxyapatite chromatography, gel exclusion chromatography, gel electrophoresis, dialysis, or affinity chromatography.

**[Para 31]** A variety of methods exist in the art for the production of monoclonal antibodies and thus, the invention is not limited to their sole production in hybridomas. For example, the monoclonal antibodies may be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567. In this context, the term "monoclonal antibody" refers to an antibody derived from a single eukaryotic, phage, or prokaryotic clone. The DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies, or such chains from human, humanized, or other sources). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transformed into

host cells such as NS0 cells, Simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; Morrison et al, *supra*) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

**[Para 32]** The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain cross-linking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent cross-linking.

**[Para 33]** Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

**[Para 34]** For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol. Methods* 125:191-202; U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety.

**[Para 35]** Humanized antibodies are antibody molecules generated in a non-human species that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework (FR) regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions

will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entireties). Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332).

**[Para 36]** Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the methods of Winter and co-workers (Jones et al., *Nature*, 321:522-525 (1986); Reichmann et al., *Nature*, 332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possible some FR residues are substituted from analogous sites in rodent antibodies.

**[Para 37]** Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety. The techniques of Cole et al., and Boerder et al., are also available for the preparation of human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Riss, (1985); and Boerner et al., *J. Immunol.*, 147(1):86-95, (1991)).

**[Para 38]** Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, Calif.), Genpharm (San Jose, Calif.), and Medarex, Inc. (Princeton, N.J.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

**[Para 39]** Also human mAbs could be made by immunizing mice transplanted with human peripheral blood leukocytes, splenocytes or bone marrows (e.g., Trioma techniques of XTL). Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of

a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

**[Para 40]** Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

**[Para 41]** Antibodies useful in the present invention may be bispecific antibodies. Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present invention, one of the binding specificities is directed towards IL13, the other may be for any other antigen, and preferably for a cell-surface protein, receptor, receptor subunit, tissue-specific antigen, virally derived protein, virally encoded envelope protein, bacterially derived protein, or bacterial surface protein, etc.

**[Para 42]** Methods for making bispecific antibodies are well known. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published May 13, 1993, and in Traunecker et al., *EMBO J.*, 10:3655-3659 (1991).

**[Para 43]** Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It may have the first heavy-chain constant

region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transformed into a suitable host organism. For further details of generating bispecific antibodies see, for example Suresh et al., *Meth. In Enzym.*, 121:210 (1986).

**[Para 44]** In addition, one can generate single-domain antibodies to IL-13. Examples of this technology have been described in WO9425591 for antibodies derived from Camelidae heavy chain Ig, as well in US20030130496 describing the isolation of single domain fully human antibodies from phage libraries.

#### IDENTIFICATION OF IL-13 ANTIBODIES

**[Para 45]** Antagonist monoclonal antibodies useful in the present invention bind IL13 and inhibit its activity, and in particular, inhibit the activation of the IL13 receptor complex. Specific antibodies useful in the present invention include 228B/C-1, 228A-4, 227-26, and 227-43, and humanized clones of these antibodies.

**[Para 46]** Candidate anti-IL13 antibodies may be tested by enzyme linked immunosorbent assay (ELISA), Western immunoblotting, or other immunochemical techniques. Assays performed to characterize the individual antibodies included: (1) Inhibition of IL13-autocrine proliferation of Hodgkin's lymphoma cell lines HDLM-2 and L-1236; (2) Inhibition of IL13-induced STAT6 phosphorylation; (3) Inhibition of IL13-induced suppression of CD14 expression in primary human monocytes; and (4) Inhibition of IL13-induced up-regulation of CD23 expression on primary human monocytes. Experimental details may be found in the Examples of application WO2004US43501.

**[Para 47]** Antibodies of the invention include, but are not limited to, monovalent, bispecific, multispecific, human, humanized or chimeric antibodies, single chain antibodies, single-domain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above.

**[Para 48]** The antibodies useful in the invention may be antigen-binding antibody fragments and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and single-domain antibodies comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments comprising any combination

of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals.

**[Para 49]** The antibodies useful in the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of IL13 or may be specific for both IL13 as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., *J. Immunol.* 147:60-69 (1991); U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., *J. Immunol.* 148:1547-1553 (1992).

**[Para 50]** Antibodies useful in the present invention may be described or specified in terms of the epitope(s) or portion(s) of IL13 which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures.

**[Para 51]** Antibodies useful in the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that bind IL13 polypeptides, which have at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to IL-13 are also included in the present invention.

**[Para 52]** Further included are antibodies that bind to the same epitope as the anti-IL13 antibodies useful in the present invention. To determine if an antibody can compete for binding to the same epitope as the epitope bound by the anti-IL13 antibodies, including the antibodies produced by the hybridomas deposited with the ATCC, a cross-blocking assay, e.g., a competitive ELISA assay, can be performed. In an exemplary competitive ELISA assay, IL13 coated on the wells of a microtiter plate is pre-incubated with or without candidate competing antibody and then the biotin-labeled anti-IL13 antibody of the invention is added. The amount of labeled anti-IL13 antibody bound to the IL13 antigen in the wells is measured using avidin-peroxidase conjugate and appropriate substrate. The antibody can be labeled with a radioactive or fluorescent label or some other detectable and measurable label. The amount of labeled anti-IL13 antibody that bound to the antigen will have an indirect correlation to the ability of the candidate competing antibody (test antibody) to compete for binding to the same epitope, i.e., the greater the affinity of the test antibody for the same epitope, the less labeled antibody will be bound to the antigen-coated wells. A candidate competing antibody is considered an antibody that binds substantially to the same epitope or that competes for binding to the same epitope as an

anti-IL13 antibody of the invention if the candidate antibody can block binding of the IL13 antibody by at least 20%, preferably by at least 20-50%, even more preferably, by at least 50% as compared to the control performed in parallel in the absence of the candidate competing antibody. It will be understood that variations of this assay can be performed to arrive at the same quantitative value.

**[Para 53]** Specific antibodies useful in the present invention include 228B/C-1, 228A-4, 227-26, and 227-43. The hybridomas that produce these antibodies were deposited on November 20, 2003, with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, under Accession Numbers PTA-5657, PTA-5656, PTA-5654, and PTA-5655, respectively. These antibodies are described in a co-pending application (WO05062972, filed 23 Dec 2004), which is incorporated herein by reference.

**[Para 54]** Antibodies useful in the present invention also include human antigen-binding antibody fragments of the antibodies including, but are not limited to, Fab, Fab' and F(ab')-2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv). The invention also includes single-domain antibodies comprising either a VL or VH domain. One example is an scFv having the sequence as set forth in SEQ ID NO 152.

**[Para 55]** Antibodies also useful in the present invention include humanized sequences of monoclonal antibody 228B/C-1. These humanized recombinant antibody molecules comprise a variable light chain region comprising an amino acid sequence having the formula: FRL1-CDRL1-FRL2-CDRL2-FRL3-CDRL3-FRL4, wherein FRL1 consists of any one of SEQ ID Nos: 20-25; CDRL1 consists of any one of SEQ ID NOs: 99-103; FRL2 consists of SEQ ID NO: 29; CDRL2 consists of any one of SEQ ID NOs: 104-114; FRL3 consists of any one of SEQ ID NOs: 30-56; CDRL3 consists of any of SEQ ID NOs: 115-116; and FRL4 consists of SEQ ID NO: 57-59; and comprising a variable heavy chain region comprising an amino acid sequence having the formula: FRH1-CDRH1-FRH2-CDRH2-FRH3-CDRH3-FRH4, wherein FRH1 consists of any one of SEQ ID NOs: 60-66; CDRH1 consists of any one of SEQ ID NOs: 117-122; FRH2 consists of any one of SEQ ID NOs: 67-75; CDRH2 consists of any one of SEQ ID NOs: 123-134; FRH3 consists of any one of SEQ ID NOs: 76-90; CDRH3 consists of any of SEQ ID NOs: 135-141; and FRH4 consists of SEQ ID NO: 91-92. The variable heavy chain region may further comprise at least the CH1 domain of a constant region or the CH1, CH2 and CH3 domains of a constant region. The heavy chain constant region may comprise an IgG antibody, wherein the IgG antibody is an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody, wherein the constant region preferably lacks effector functions, e.g., ADCC or CDC.

**[Para 56]** Additionally, antibodies included comprise recombinant antibody molecules wherein the variable light chain is chosen from any one of SEQ ID Nos: 3, 5, 7, 93, 95, 97, 142, 144, and 150, and a variable heavy chain chosen from any one of SEQ ID Nos: 4, 6, 8, 94, 96, 98, 143, 145, 146, 147, 148, and 149. One particular antibody comprises the variable light chain having the sequence set forth in SEQ ID NO:142, and a variable heavy chain having the sequence set forth in SEQ ID NO:143.

**[Para 57]** The binding epitope of MAb 228B/C-1 was mapped to a unique site on IL13 responsible for the interaction with IL4R $\alpha$ , which constitutes part of the multimeric IL13R complex.

#### METHODS OF PRODUCING ANTI-IL13 ANTIBODIES

**[Para 58]** Anti-IL13 antibodies may be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

**[Para 59]** Recombinant expression of an antibody, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody or a single chain antibody), requires construction of an expression vector containing a polynucleotide that encodes the antibody or a fragment of the antibody. Once a polynucleotide encoding an antibody molecule has been obtained or constructed, the vector for the production of the antibody may be produced by recombinant DNA technology. An expression vector is constructed containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. These techniques are conventional in the art and may be found, e.g., in Current Protocols in Molecular Biology, Ed. Fred M. Ausubel et al., ISBN: 0-471-50338-X). The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured to produce an antibody. In one aspect of the invention, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

**[Para 60]** A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention as described above. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. Bacterial cells such as E. coli, and eukaryotic cells are commonly used for the expression of a recombinant antibody molecule, especially for the expression of

whole recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

**[Para 61]** In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, COS, 293, 3T3, or myeloma cells.

**[Para 62]** For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

**[Para 63]** A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., *Cell* 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA* 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., *Cell* 22:817 (1980)) genes can be employed in tk, hgprt or aprt-cells, respectively. Also, antimetabolite

resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Proc. Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, Biotherapy 3:87-95 (1991)); and hygromycin, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

**[Para 64]** The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, "The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells" (DNA Cloning, Vol.3. Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

**[Para 65]** The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

**[Para 66]** Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific

antigen after Protein A, and size-exclusion chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies useful in the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

**[Para 67]** The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide. Fused or conjugated antibodies useful in the present invention may be used for ease in purification. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Pat. No. 5,474,981; Gillies et al., *Proc. Natl. Acad. Sci.* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452(1991), which are incorporated by reference in their entireties.

**[Para 68]** Moreover, the antibodies or fragments thereof useful in the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)) and the "flag" tag.

**[Para 69]** The amount of the antibody which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of IL13 can be determined by standard clinical techniques. The antibody can be administered in treatment regimes consistent with the disease, e.g., a single or a few doses over one to several days to ameliorate a disease state or periodic doses over an extended time to prevent allergy or asthma. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

**[Para 70]** For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to

10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

[Para 71] The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3, IL-7, IFN, GCSF, GMCSF, Flt3, IL21) and unmethylated CpG containing oligonucleotides, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

#### THERAPEUTIC ADMINISTRATION

[Para 72] In the present invention, an anti-IL13 antibody can be administered to the mammal in any acceptable manner. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, inhalation and oral routes. The antibodies or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the therapeutic antibodies or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

[Para 73] Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. The antibody may also be administered into the lungs of a patient in the form of a dry powder composition (See e.g., U.S. Pat. No. 6,514,496).

[Para 74] In a specific embodiment, it may be desirable to administer the anti-IL13 antibody or a composition thereof locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering an

antibody of the invention, care must be taken to use materials to which the protein does not absorb.

**[Para 75]** In another embodiment, the antibody can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*).

**[Para 76]** In yet another embodiment, the antibody can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target.

**[Para 77]** The present invention also provides for the administration of pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of the antibody, and a physiologically acceptable carrier. In a specific embodiment, the term "physiologically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such physiological carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release

formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain an effective amount of the antibody, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

**[Para 78]** In one embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

**[Para 79]** The invention also provides for a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention useful in the treatment of fibrosis. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

#### EXAMPLES

**[Para 80]** This invention can be further illustrated by the following examples of preferred embodiments thereof, although it will be understood that these examples are included merely for purposes of illustration and are not intended to limit the scope of the invention unless otherwise specifically indicated.

**[Para 81]** Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the

invention described herein. Such equivalents are intended to be encompassed by the claims.

**Example 1: Mouse Model for Chemotherapy-induced Pulmonary Fibrosis**

**[Para 82]** Female CBA/J mice (6-8 wk old; the Jackson Laboratory, Bar Harbor, ME) are used. Mice are maintained under pathogen-free conditions and provided with food and water ad libitum. To induce pulmonary fibrosis, mice are treated on Day Zero intratracheally with a chemotherapy agent, such as bleomycin (See Keane MP. J Immunol. (1999) 162: 5511-5518). Control animals receive sterile saline instead of the agent.

**[Para 83]** Briefly, the mice are anesthetized with 250  $\mu$ L of 12.5  $\mu$ g/mL ketamine injected intraperitoneally followed by intratracheal instillation of 0.025 U of bleomycin in 25  $\mu$ L of sterile isotonic saline. To study the effect of inhibition of IL13 on bleomycin-induced pulmonary fibrosis, bleomycin-treated mice (30 per group) are treated intraperitoneally with an anti-mouse IL13 antibody at 1 or 10 mg/kg on a day before bleomycin treatment and on day 3, day 7, day 11, day 15 and day 18 after bleomycin treatment. For control, 30 bleomycin-treated mice are administered the same doses of an irrelevant isotype-matched control antibody. On day 2, 4, 8, 12, 16 and 20 days post-treatment, 6 animals are euthanized at each time point. Both lungs are removed for analysis of fibrosis by hydroxyproline assay (See Example 3 below) and histology.

**EXAMPLE 2: Mouse Model for Radiation-induced Pulmonary Fibrosis**

**[Para 84]** C3H mice are given whole lung irradiation of either 14 (LD50 of C3H mice) or 16 Gy (LD100 of C3H mice) and then sacrificed when moribund or at 12 weeks according to the protocol described by Haston CK. (Cancer Res. 2002; 62: 3782-4788). The mice are treated with either an anti-IL13 antibody or irrelevant isotype-matched control. Both lungs are removed for analysis of fibrosis by hydroxyproline assay and histology.

**EXAMPLE 3: Hydroxyproline Assay for Collagen Deposition**

**[Para 85]** Total lung collagen is determined by analysis of hydroxyproline as previous described (Keane MP. J Immunol. 1999; 163: 5686-5692). Briefly, lungs are harvested and homogenized in 2 mL of phosphate-buffered saline, pH 7.4. One-half milliliter of each sample (left lungs) is digested in 1 mL of 6 N HCl for 8 h at 120°C. Five microliters of citrate/acetate buffer (5% citric acid, 7.24% sodium acetate, 3.4% sodium hydroxide, and 1.2% glacial acetic acid, pH 6.0) and 100  $\mu$ L of chloramines-T solution (282 mg of chloramines-T, 2 mL of n-propanol, 2 mL of H<sub>2</sub>O, and 16 mL of citrate/acetate buffer) are added to 5  $\mu$ L of sample, and the samples left at room temperature for 20 min. Next 100  $\mu$ L of Ehrlich's solution (2.5 g of 4-(dimethylamino) benzaldehyde (Aldrich, Milwaukee, WI), 9.3 mL of n-propanol, and 3.9 mL of 70% perchloric acid (Eastman Kodak,

Rochester, NY) are added to each sample, and the samples are incubated for 15 min at 65°C. Samples are cooled for 10 min and read at 550 nm on a spectrophotometer.

Hydroxylproline (Sigma, St. Louis, MO) concentrations from 0-10 µg/mL is used to construct a standard curve.

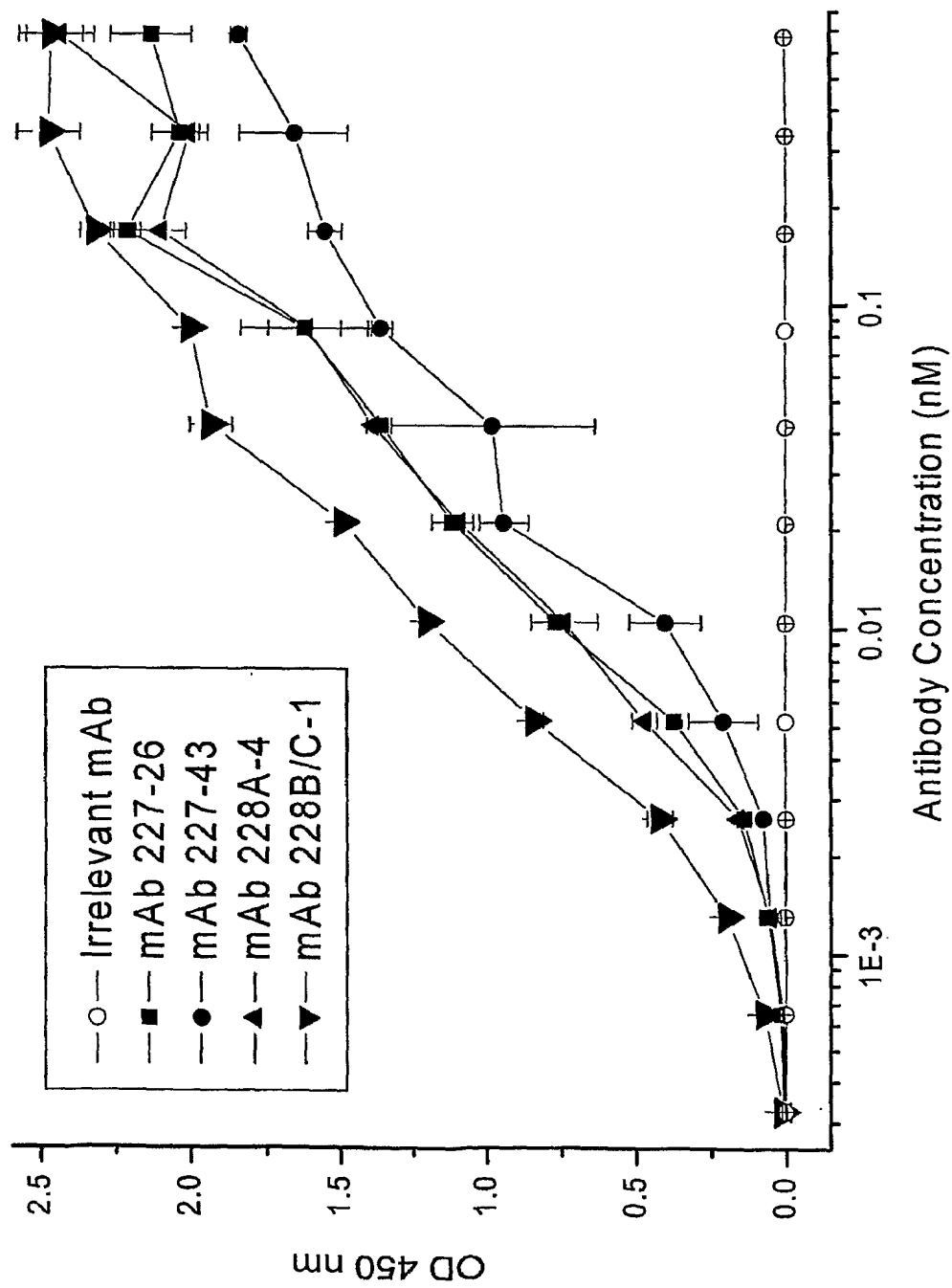
**[Para 86]** The right lung from each animal is used for histological studies by inflating them with 10% formalin solution instilled through the trachea, and fixed for 24 h. After embedding the lungs in paraffin, sections are prepared and stained with hematoxylin and eosin (H&E) or Masson's trichrome. Lungs are examined for the presence of inflammatory infiltrates and interstitial pulmonary fibrosis. For the purpose of quantitation, fibrosis is defined as areas that have Ashcroft grade 7 or 8 (Ashcroft T. J Clin Pathol. 1988; 41: 467-70). These grades represent severe distortion of structure and large fibrous areas, including honeycomb lung, or total fibrous obliteration of the field. Results are expressed as square pixels at x400 magnification using a digital imaging system coupled to a microscope.

**We Claim:**

1. A method of reducing IL13 mediated cell proliferation, cell activation or extracellular matrix production in a mammal comprising administering to the mammal a composition comprising an anti-IL13 antibody in an amount sufficient to reduce IL13 mediated cell proliferation or extracellular matrix production.
2. A method of treating fibrosis in a patient undergoing chemotherapy and/or radiotherapy comprising administering a composition comprising an anti-IL13 antibody in an amount sufficient to prevent or reduce fibrosis in said patient.
3. A method for improving radiotherapy and/or chemo-therapy comprising reducing IL13-mediated cell proliferation, cell activation or extracellular matrix production in a mammal comprising administering to the mammal a composition comprising an anti-IL13 antibody in combination with a pharmaceutically acceptable delivery vehicle, in an amount sufficient to reduce IL13 mediated cell proliferation, cell activation or extracellular matrix production and allow higher doses of radiotherapy and/or chemotherapy.
4. A method of reducing the morbidity or mortality associated with fibrosis in patients undergoing chemotherapy and/or radiotherapy comprising administering a composition comprising an anti-IL13 antibody in an amount sufficient to prevent or reduce fibrosis in said patients.
5. The method according to any one of claims 1 to 4, wherein proliferation or activation of smooth muscle cells, epithelial cells, endothelial cells or fibroblasts is reduced.
6. A method according to any one of claims 1 to 4, wherein the antibody neutralizes human IL13 activity at an approximate molar ratio of 1:2 (mAb:IL13).
7. The method according to any one of claims 1 to 4, wherein the antibody is monoclonal.
8. The method according to any one of claims 1 to 4, wherein the antibody is a single-domain antibody.
9. The method according to any one of claims 1 to 4, wherein the antibody is a human antibody, a chimeric antibody, or a humanized antibody.
10. The method according to claim to any one of claims 1 to 4, wherein the composition comprises the antibody and a physiologically acceptable carrier, diluent, excipient, or stabilizer.

11. The method according to any one of claims 1 to 4, wherein the antibody is administered by one or more of the routes selected from the group consisting of intravenous, intraperitoneal, inhalation, intramuscular, subcutaneous and oral.
12. The method according to any one of claims 1-4, wherein the antibody is MAb228B/C produced by the hybridoma designated PTA-5657.
13. The method according to any one of claims 1 to 4, wherein the antibody binds to the same epitope as the antibody 228B/C-1 produced by the hybridoma designated PTA-5657.
14. The method according to any one of claims 1 to 4, wherein the antibody is MAb228A-4 produced by the hybridoma designated PTA-5656; MAb227-26 produced by the hybridoma designated PTA-5654; or MAb227-43 produced by the hybridoma designated PTA-5655.
15. The method of claim 1, wherein the antibody comprises at least a CDRH3 of antibody 228B/C-1 produced by the hybridoma designated PTA-5657.
16. The method of claim 1, wherein said antibody has a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 3, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 4.
17. The method of claim 1, wherein said antibody has a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 5, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 6.
18. The method of claim 1, wherein said antibody has a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 7, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 8.
19. The method according to claim 1, wherein the antibody, or an IL13-binding fragment thereof, comprises a heavy chain comprising SEQ ID NO 135, 136, 137, 138, 139, 140 or 141.
20. The method of claim 1, wherein the antibody comprises a variable light chain region having a CDRL1 chosen from SEQ ID NOs: 99-103; a CDRL2 chosen from SEQ ID Nos: 104-114; and a CDRL3 chosen from SEQ ID NOs: 115-116.
21. The method of claim 1, wherein the antibody comprises a variable light chain region having the formula: FRL1-CDRL1-FRL2-CDRL2-FRL3-CDRL3-FRL4, wherein FRL1 comprises any one of SEQ ID Nos: 20-25; CDRL1 comprises any one of SEQ ID NOs: 99-103; FRL2 comprises SEQ ID NO: 29; CDRL2 comprises any one of SEQ ID Nos: 104-114; FRL3 comprises any one of SEQ ID NOs: 30-

- 56; CDRL3 comprises any of SEQ ID NOs: 115-116; and FRL4 comprises any one of SEQ ID NOs: 57-59.
22. The method of claim 1, wherein the antibody comprises a variable light chain region comprising any one of SEQ ID NOs: 3, 5, 7, 93, 95, 97, 142, 144, and 150.
23. The method of claim 1, wherein the antibody comprises a variable heavy chain region having a CDRH1 chosen from SEQ ID NOs: 117-122; a CDRH2 chosen from SEQ ID Nos: 123-134; and a CDRH3 chosen from SEQ ID NOs: 135-141.
24. The method of claim 1, wherein the antibody comprises a variable heavy chain region having the formula: FRH1-CDRH1-FRH2-CDRH2-FRH3-CDRH3-FRH4, wherein FRH1 comprises any one of SEQ ID NOs: 60-66; CDRH1 comprises any one of SEQ ID NOs: 117-122; FRH2 comprises any one of SEQ ID NOs: 67-75; CDRH2 comprises any one of SEQ ID NOs: 123-134; FRH3 comprises any one of SEQ ID NOs: 76-90; CDRH3 comprises any of SEQ ID NOs: 135-141; and FRH4 comprises any one of SEQ ID NO: 91-92.
25. The method of claim 1, wherein the antibody comprises a variable heavy chain region comprising any one of SEQ ID NOs: 4, 6, 8, 94, 96, 98, 143, 145, 146, 147, 148, and 149.
26. The method of claim 25, wherein the antibody is an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody, wherein the antibody lacks effector function.
27. The method of claim 20, wherein the antibody further comprises the variable heavy chain region of claim 23.
28. The method of claim 22, wherein the antibody further comprises the heavy chain of claim 25.
29. The method of claim 28, wherein the antibody comprises a variable light chain region having the amino acid sequence set forth in SEQ ID NO: 142 and a variable heavy chain region having the amino acid sequence set forth in SEQ ID NO: 143.
30. The antibody of claim 28, wherein the antibody comprises a variable light chain region having the amino acid sequence set forth in SEQ ID NO: 150 and a variable heavy chain region having the amino acid sequence set forth in SEQ ID NO: 151.
31. The method of claim 1, wherein the antibody is a single chain antibody having the sequence set forth in SEQ ID NO:152.

**Fig. 1 Binding of anti-IL13 mAbs to human IL-13 in ELISA**

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 5/06, 5/16 (2007.10)

USPC - 435/330

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
USPC: 435/330Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
USPC: 435/7.23, 344 (see search terms below)Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
PubWest: PubWest: PGPB,USPT,USOC,EPAB,JPAB; Google Scholar; Google Patents; Text: Anti-IL-13; antibody; monoclonal; anti-interleukin-13; cell proliferation; cell activation; extracellular matrix; reduce (-ing); inhibit (-ing); administer (-ing); mammal; human; fibrosis; cancer; chemotherapy (-eitic); pharmaceutically;

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/0073148 A1 (TCHISTIAKOVA et al.) 06 April 2006 (06.04.2006) para [0029]-[0030], [0093], [0096], [0145], [0190], [0208], [0213], [0235], [0247]	1-5 and 7-11
Y	WO 2005/062967 A2 (FUNG et al.) 14 July 2005 (14.07.2005) pg. 3, para [0010]-[0011]; pg. 4, para [0012]; pg. 5, para [0017]; pg 41, para [0205]; Fig. 8	6, 12-16
Y		6, 12-16

☐ Further documents are listed in the continuation of Box C.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

27 December 2006 (27.12.2006)

Date of mailing of the international search report

03 MAR 2008

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300  
PCT OSP: 571-272-7774

**Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of:
- a. type of material
- ☒ a sequence listing
- ☐ table(s) related to the sequence listing
- b. format of material
- ☒ on paper
- ☐ in electronic form
- c. time of filing/furnishing
- ☒ contained in the international application as filed
- ☐ filed together with the international application in electronic form
- ☐ furnished subsequently to this Authority for the purposes of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

Group 1: claims 1-16, having SEQ ID Nos: 3 and 4.

Group 2: claims 1 and 17, limited to SEQ ID Nos: 5 and 6.

Group 3: claims 1 and 18, limited to SEQ ID Nos: 7 and 8.

Group 4-10: claims 1 and 19, limited to one of each SEQ ID Nos: 135, 136, 137, 138, 139, 140 or 141.

Groups 11-28: claims 1 and 20, limited to one variable light chain region having a CDRL1 chosen from one of each SEQ ID Nos: 99-103; a CDRL2 chosen from one of each SEQ ID Nos: 104-114; and, a CDRL3 chosen from one of each SEQ ID Nos: 115-116.

Groups 29-83: claims 1 and 21, further limited wherein FRL1 comprises any one of SEQ ID Nos: 20-25; CDRL1 comprises any one of SEQ ID Nos: 99-103; FRL2 comprises SEQ ID No: 29; CDRL2 comprises any one of SEQ ID Nos: 104-114; FRL3 comprises any one of SEQ ID Nos: 30-56; CDRL3 comprises any of SEQ ID Nos: 115-116; and FRL4 comprises any one of SEQ ID Nos: 57-59.

Groups 84-92: claims 1 and 22, further limited wherein the antibody comprises a variable light chain region comprising any one of SEQ ID Nos: 3, 5, 7, 93, 95, 97, 142, 144, and 150.

Groups 93-117: claims 1 and 23, further limited wherein CDRH1 is chosen from any one of SEQ ID Nos: 117-122; CDRH2 is chosen from any of SEQ ID Nos: 123-134; and a CDRH3 is chosen from any one of SEQ ID Nos: 135-141.

Groups 118-175: claims 1 and 24, wherein FRH1 comprises any one of SEQ ID Nos: 60-66; CDRH1 is selected from any one of SEQ ID Nos: 117-122; FRH2 comprises any one of SEQ ID Nos: 67-75; CDRH2 comprises any one of SEQ ID Nos: 123-134; FRH3 comprises any one of SEQ ID Nos: 76-90; CDRH3 comprises any one of SEQ ID Nos: 135-141; and FRH4 comprises any one of SEQ ID Nos: 91-92.

Groups 176-187: claims 1 and 25-26, wherein the antibody comprises a variable heavy chain region comprising any one of SEQ ID Nos: 4, 6, 8, 94, 96, 98, 143, 145, 146, 147, 148, and 149.

Groups 188: claims 1 and 31, wherein the antibody is a single chain antibody having the sequence set forth in SEQ ID No: 152.

The inventions listed as groups 1-188 above do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2 they lack the same or corresponding special technical feature. According to PCT Rule 13.2, unity of invention exists only when the same or corresponding technical feature is shared by all claimed inventions.

Groups 1-188 of this application contain claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

These species are amino acids having SEQ ID Nos: 3-8, 20-25, 29-150 and 152.

SEQ ID Nos: 3-8, 20-25, 29-150 and 152 do not share any significant structural element and cannot be considered as having the same or corresponding technical feature.

The different physical characteristics of the sequences represent distinct traits that are measured using different techniques and compared using different criteria. The different polypeptide sequences represented by the amino acid content of the species are different structures that are not common to one another but are different because they are composed of unique amino acid sequences.

In this case the first named invention and first named species that will be searched without additional fees is Group 1 represented by claims 1-16, having SEQ ID Nos: 3 and 4.

In order for more than one species to be examined, the appropriate additional examination fees must be paid and the desired species clearly identified.

Claims 27-30 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 27-30  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:  
see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-16.

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.